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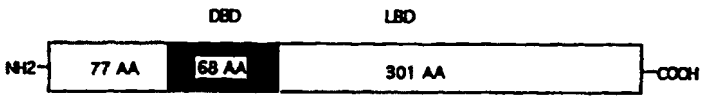

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h ARP-1	< 10 %	51.1 %	30 %
h COUP-TF	< 10 %	51.1 %	29 %
r VDR	< 10 %	50 %	34.7 %
h RXR α	< 10 %	50 %	23.6 %
r TR β	< 10 %	50 %	29.7 %
r PPAR	< 10 %	50 %	28.5 %
r MR	< 10 %	50 %	18.3 %
r NGF-1	< 10 %	48.5 %	30.3 %

(57) Abstract

This invention provides an isolated receptor having the amino acid sequence of Fig. 1 or substantially the same amino acid sequence as the amino acid sequence shown in Fig. 1 or an amino acid sequence functionally similar to that sequence, and DNA sequences encoding such a receptor.

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OR-1 ON ORPHAN RECEPTOR BELONGING TO THE NUCLEAR RECEPTOR FAMILY

This invention relates to cellular nuclear receptors.

5 A large family of nuclear receptors has been identified which confer cells with responsiveness to molecules such as retinoic acid, vitamin D3, steroid hormones and thyroid hormones. Extensive studies have shown that the members of this superfamily of nuclear receptors activate and/or repress gene transcription through direct binding to discrete *cis*- acting elements termed "hormone response elements" (HRE). It has been 10 shown that these HRE's comprise repeats of consensus palindromic hexanucleotide DNA motifs. The specificity of the HRE's is determined by the orientation of, and spacing between, halfsites (i.e. half a palindromic sequence)(Umenesono K.,*et al*, 1991 *Cell* **65**, 1255-1266).

15 Specific DNA binding is mediated by a distinct DNA binding domain, containing two zinc fingers, which is conserved among all thus discovered nuclear receptors. Three amino acids at the C-terminal base of the first zinc finger (known as the "P-box") are important for the recognition of the half site nucleotide sequence. Members of the nuclear receptor superfamily have been classified into different groups on the basis of the amino acid 20 sequence within the P box.

Molecules thought to be nuclear receptors, as they are structurally related to characterized receptors, but for which no ligand has been identified are termed "orphan receptors". Many such orphan receptors have been identified (see for example Evans R.M, (1988) *Science* **240**,889-895 and O'Malley, B. (1990) *Mol. Endocrinol.* **4** 363-369).

According to one aspect of the invention there is provided a novel nuclear receptor, hereinafter termed "OR-1", having the amino acid sequence of Figure 1 or substantially the same amino acid sequence as the amino acid sequence shown in Fig. 1 or an amino acid sequence functionally similar to that sequence.

An amino acid sequence which is more than about 90%, preferably more than 95%, identical with the sequence shown in Fig. 1 is substantially the same amino acid sequence for the purposes of the present application.

According to another aspect of the invention there is provided a DNA sequence encoding a nuclear receptor according to the first aspect of the invention. Preferably, the DNA sequence is that given in Fig. 2 or is a DNA sequence encoding a protein or polypeptide having the functionality of OR-1.

The nuclear receptor of the invention has a similar P-box configuration to the retinoic acid receptor (RAR), the vitamin D receptor (VDR), and the thyroid hormone receptor (TR) and can be placed in the same subfamily as those receptors.

Preferably, the receptor heterodimerizes with RXR to form a complex.

Preferably, the receptor interacts with RXR and binds to a DNA sequence comprising at least one repeat of the DNA sequence -AGGTCA- . Preferably the sequence is AGTCAGGTCACTCGAGGTCAGTCA.

Preferably, the receptor modulates 9-*cis* retinoic acid signalling.

The nuclear receptor of the invention, OR-1, and its method of production will now be described, by way of example only, with reference to the accompanying drawings Figures 1 -5, in which :

Fig. 1 shows the amino acid sequence of a nuclear receptor of the invention;

Fig. 2 shows the DNA sequence of a nuclear receptor of the invention;

Fig. 3 gives a comparison between the primary amino acid sequences of the nuclear receptor of the invention and those of other members of the nuclear receptor superfamily;

Fig. 4 Localization of OR-1 mRNA - producing cells in rat tissues with in situ hybridization;

Fig. 5A gives the DNA sequences of seven potential HRE's DR-0 - DR-6;

Fig. 5B illustrates the interaction between OR-1 or the retinoid X receptor (RXR) and the potential HRE's, DR-2 and DR4; and

Fig. 6 illustrates experiments showing that OR-1 confers 9-*cis* retinoic acid-responsiveness of RXR on a DR-4 -containing promoter.

CLONING AND EXPRESSION OF OR-1

Rat OR-1 was cloned from a cDNA library from Sprague Dawley rat liver in the commercially-available λZAP vector (Stratagene, USA) using the techniques described in Göttlicher, M. *et al* (1992) *Proc. Natl. Acad. Sci. USA* **89**, 4653-4657.

Foetal and adult rat tissues were excised after decapitation and frozen on dry ice. Cryostat sections were hybridized to 48-mer oligonucleotides complementary to OR-1 mRNA positions 100-151 and 850-900 as described in Dagerlind, Å et al (1992) *Histochemistry* **98** 34-49.

Several unrelated oligonucleotides were also used as controls. The addition of 100 fold of the respective nonlabelled control oligonucleotide abolished all labelling observed with the OR-1 probes.

PLASMIDS

OR-1 cDNA was subcloned as an Eco RI fragment in pGEM-3Z (Promega) to produce the plasmid pROR-1-Sp6, or in the multiple cloning site of pCMV5 (described in Andersson, S. *et al* 1989 *J. Biol. Chem.*, **264**, 8222-8229) to produce the plasmid pCMV-OR-1. The reporter construct pDR4-AF contains a SphI-XhoI fragment of the cDNA for a secreted

form of human PAP (placental alkaline phosphatase) described in (Berger, J. *et al.*1988 *Gene* **66**,1-10) under the control of a DR4-TK-containing promoter, pRRXR-T7 and pCMV-RXR described previously in Gearing,K.L. *et al* 1993 *Proc. Natl. Acad. Sci. USA* **90**, 1440-1444.

DNA BINDING STUDIES

Gel shifts were performed using *in vitro*-translated OR-1 and RXR with the commercially-available TNT™-coupled reticulocyte lysate system (Promega, Madison USA). Proteins were incubated on ice for 15 min with 4μg of poly (dI-dC) and with unlabelled competitor DNA where indicated in a solution comprising 100mM KCl; 10mM Hepes,pH7.6; 1mM dithiothreitol; 1 mM EDTA; 10% (wt./vol) glycerol, before addition of 0.5 ng of a ^{32}P-end labelled oligonucleotide probe. The reaction mixtures were incubated for a further 10 min at 22° C before electrophoresis at 200V and 4 °C in pre-run 4 % polyacryliamide/ 0.25 TBE (0.089m tris-borate pH 8.3, 0.025 EDTA) gels.

The following oligonucleotides and their complements were used as probes:

DR0 AGCTTCAGGTCAAGGTCAGGTTCA

DR1 AGCTTCAGGTCACAGGTCAGTTCA

DR2 AGCTTAGGTCACCAGGTCAGTTCA

DR3 AGTCCAGGTCACTCAGGTCAGTCA

DR4 AGTCAGGTCACTCGAGGTCAGTCA

DR5 AGTCAGGTCACTCGTAGGTCAGTCA

DR6 AGTCAGGTCACTCGTTAGGTCAGTCA

CELLS AND TRANSFECTION

Embryonal carcinoma P19 EC cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% foetal calf serum, nonessential amino acids, penicillin (100 units/ml) and streptomycin (100mg/ml). Chinese Hamster Ovary (CHO) cells were cultured in Ham's F-12 medium supplemented with 10% foetal calf serum, penicillin (100 units/ml) and streptomycin (100 mg/ml). Cells were plated in duplicate in 35mm Petri dishes and transfected at 30% confluency, using lipofectin reagent (Bethesada Research Laboratories, USA) according to the recommendations of the supplier. After 12 hours the medium was changed and supplemented or not supplemented as the case may be with 100nM 9-*cis* retinoic acid (a gift of Hoffman-LaRoche) as indicated, and incubated for an additional 36h. Cell culture supernatants were then heated to 65°C for 30min. PAP activity was determined as the increase in A_{405} at 30°C in a 1 ml reaction mixture containing 0.75ml of supernatant, 200nM Tris (pH8.8.), 275 mM NaCl, 0.5 mM $MgCl_2$, and 5mM p-nitrophenylphosphate.

Transfections were repeated 6 times with different plasmid preparations and data from a representative experiment is presented here.

RESULTS

The OR-1 clone spans 1940bp including a 55bp long poly-A tail and contains an open reading frame starting with an ATG corresponding to a protein of 446 amino acids with a predicted molecular weight of 50kD. The complete amino acid and nucleotide sequences of OR-1 are given in Fig. 1 and 2 respectively. OR-1 shows no striking homology to known members of the nuclear receptors superfamily: the highest homologies represent less than

10% in the N-terminal domain, about 50% in the DNA binding domain, and between 20-30 % in the putative ligand binding domain as shown in Fig.3.

The amino-terminal domain of OR-1 (underlined in Fig. 1) is 77 amino acids long and to a large extent comprises a so-called "PEST" sequence, meaning that it is an amino acid sequence rich in proline, glutamic acid, serine, threonine, and aspartic acid residues.

The DNA binding domain consists of 68 amino acids including the nine invariable cysteines characteristic of the members of the nuclear receptor superfamily, as well as other amino acids that are found to be conserved in all members of this protein family.

GENOMIC CLONING

A rat genomic fragment has been isolated, that spans the DNA binding domain or OR1 and all the exons downstream of it. Most nuclear receptors for which the genomic structure has been determined have the two zinc "fingers" of the DNA binding domain encoded on separate exons. We have shown that the whole DNA-binding domain is encoded by one exon in OR1. We have furthermore shown that this is also the case with RLD-1 (*Mol. Endocrinol. infra*), a closely related receptor "knock-out" mice of OR1 and RLD-1.

TISSUE DISTRIBUTION OF OR-1

To analyse the tissue distribution of OR-1 transcripts, *in situ* hybridizations were performed on foetal and adult rat tissues. Labelling for OR-1 was found in several tissues of both foetal and adult rats. As discussed, below, prominent expression was observed in liver, lung, thymus, brown fat, salivary gland, thyroid gland, pituitary gland and retina whereas moderate levels were seen in developing cerebrum and cerebellum, in

perichondrium around developing bones, heart and skin. Low levels of OR-1 mRNA was present in skeletal muscle as shown in Fig. 4. In adult rats, strong labelling was found in lymph node, prostate, adrenal cortex and the intermediate lobe of the pituitary gland. Moderate levels were seen in liver, testis, salivary gland, thyroid and parathyroid gland, adrenal medulla, anterior pituitary and kidney. In the brain, a moderate signal was observed in neurons in the granular cell layer of the cerebellum and hippocampus.

1) IMMUNE SYSTEM

Prominent expression of OR-1 mRNA was seen in the cortex of the thymus with lower levels in the medulla. In dipped sections grains were seen over most of the thymocytes in the cortex. Significant expression was also seen in the lymph nodes, whereas low levels were observed in spleen. Some cells in the bone marrow expressed OR-1 mRNA.

2) ENDOCRINE SYSTEM

Significant expression of OR-1 was seen in the anterior and intermediate lobes of the pituitary. In dipped sections grains could be seen over most of the cells in the intermediate lobe and over the majority of the cells in the anterior lobe. The posterior lobe appeared virtually nonlabelled. Prominent expression of OR-1 was detected in the parathyroid glands where most of the cells expressed OR-1 mRNA. In the thyroid gland moderate expression was observed and OR-1 mRNA was heterogenously distributed in different cell types. Most of the parafollicular cells expressed OR-1, whereas only part of the follicular cells were labelled.

High expression in the adrenal gland was observed in all layers of the cortex, whereas lower levels were seen in the medulla. Expression of OR-1 was slightly higher in the zona glomerulosa than in the rest of the cortex. In the adrenal medulla the labelling was heterogenous and part of the chromaffin cells and ganglion cells expressed OR-1. In pineal gland some cells contained OR-1 mRNA.

3) **REPRODUCTIVE SYSTEM**

OR-1 could be detected both in male and remale genital organs. In the testis OR-1 mRNA was present in all cross-sections of the seminiferous tubules. The labelling localizes to the basal compartment of the seminiferous epithelium and grains could be seen mainly over primary spermatocytes, whereas spermatogonia and germ cells at later developmental stages were non-labelled. The Sertoli cells and Leydig cells did not express OR-1 mRNA. A strong signal for OR-1 was evident in the epithelium of the prostate gland and also in the epididymis, whereas low levels were seen in the epithelium of the vesicula seminalis. In the ovary oocytes at different stages of development expressed OR-1 mRNA while other cells appeared non-labelled. In the uterus the epithelium was strongly labelled and lower leves of OR-1 mRNA were seen in the myometrium.

4) **URINARY SYSTEM**

Moderate expression of OR-1 could be detected in the outer medulla of the

kidney, whereas in the cortex and inner medulla the labelling was very low or nondetectable. In dipped sections grains were seen over different parts of the loop of Henle. The glomeruli, proximal and distal convoluted tubules and collecting tubules did not express OR-1 at detectable levels. The transitional epithelium of the renal pelvis expressed OR-1.

5) **DIGESTIVE SYSTEM**

In salivary glands the secretory acini and the ducts expressed moderate levels of OR-1 mRNA. In the liver OR-1 mRNA was evenly distributed throughout the liver and most, if not all, hepatocytes were labelled. In the intestinal tract OR-1 was expressed in the epithelium of stomach and small and large intestine.

6) **NERVOUS SYSTEM**

Significant expression of OR-1 was seen in the sympathetic and sensory ganglia. In superior cervical ganglion most of the sympatetic neurons expressed OR-1 at high level and also the satellite cells were labelled. In dorsal root ganglion the labelling was heterogenous and varied between individual neurons. The Schwan cells of peripheral nerves expressed OR-1 whereas oligodendrocytes in optic nerve were nonlabelled. In the retina the bipolar cells expressed OR-1. In the central nervous system OR-1 mRNA was seen in several areas including hippocampus and cerebellum.

7) **RESPIRATORY SYSTEM**

Moderate expression of OR-1 was seen in the bronchial epithelium and in the alveoli.

8) **OTHER TISSUES**

Low or non-detectable levels of OR-1 were seen in sketal muscle and heart. Also in white adipose tissue OR-1 expression was below the detection limit. In skin a clear signal was observed in keratinocytes in the basal part of the epidermis. A strong signal was seen in perichondrium around the cartilage in trachea. Low expression of OR-1 could be seen in intra and extraorbital lacrimal glands.

The expression of OR-1 thus appears to be ubiquitous, suggesting that this receptor might have a house keeping function and/or mediate many effects by regulating the transcriptor of various genes. The tissue distribution or OR-1 is different from the tissue distribution of RLD-1 (*Mol Endocrinol* **9**, 72-85, 1995) suggesting that these two isoforms might have different functions. OR-1 is particularly well expressed in tissues involved in the immue system. It has been described that 9-cis retinoic acid plays a role in thymocyte development, being a potent negative regulator of activation-induced T-cell apoptosis. Since OR-1 dimerizes with RXR and is expressed at a high level in the thymus during the fetal stages, it may play a role in regulating T-cells development. OR-1 is also well expressed in peripheral

endocrine glands, in male and female genital organs and in the nervous system. The tissue distribution of OR-1 is thus different from that of RXRα which has been described to be noticeably abundant in visceral tissues such as liver, kidney, lung, brain, heart, intestine and testis. We previously suggested that OR-1 could act as a helper of RXRα in mediating the effects of 9-cis retinoic acid. Nevertheless we do not know whether OR-1 could also act as a monomer, as a homodimer or as a heterodimer with another protein than RXRα. For example, it is possible that OR-1 modulates the actions of RXRβ that shows a diffuse and probably ubiouitous expression, and of RXRγ which has a more specific distribution.

OR-1 INTERACTS WITH RXR ON A DR4 MOTIF *IN VITRO*

A set of potential HRE's, DR0-DR6, having the DNA sequences described above predicted by the 3-4-5 rule (Umensono *et al supra*) was synthesized and assayed in gel shift experiments using *in vitro* translated 0R-1 alone or in combination with RXR also translated *in vitro*. *In vitro* translation of OR-1 produced a protein of the predicted size of 50kD. In the gel shift assays, OR-1 was unable to bind to any of the potential HRE's but OR-1 combined with RXR, recognized the potential HRE DR4 which is usually described as the thyroid hormone response element (TRE)(Umensono *et al supra*).

Fig. 5B shows that although OR-1 or RXR alone was not able to bind to DR4, together these proteins were able to form a specific complex with this DNA element. The appearance of this complex depends on the presence of RXR and is inhibited by a 10-fold

excess of the specific DNA target element, but not by a 100-fold excess of an unrelated DNA element - see Fig. 5B, lane 7)

OR-1 CONFERS 9-*CIS* RETINOIC ACID RESPONSIVENESS OF RXR ON A DR4-CONTAINING PROMOTER

Since OR-1 and RXR formed a specific complex on the DR4 sequence *in vitro*, coexpression of OR-1 in embryonal carcinoma (EC) cells that express endogenous RXR was tested to determine whether it could affect the activity of a reporter gene under the control of a DR4-containing promoter. RXR has been shown to be an auxiliary receptor for several classes of hormone receptors, controlling the ligand responses of receptors that form heterodimers with RXR (Yu, V.C. *et al* 1991 *Cell* 67, 251-1266 and Bugge, T.H. *et al* 1992 *EMBO J.* 11, 1409-1418). In addition, it has been shown that 9-*cis* retinoic acid leads to effective RXR homodimer formation and that these homodimers bind and activate several retinoic acid response elements ("RARE's"), but not natural thyroid hormone response elements (Zhang, X.K. *et al* 1992 *Nature (London)358, 587-591)*. As previously described by others (Hallenbeck, P.L. *et al* 1993, *J. Biol Chem.268, 3825-3828)* our transfection studies showed no induction by 9-*cis* retinoic acid of RXR on a reporter containing DR4 (Fig 5). Expression of OR-1 allowed activation of RXR by 9-*cis* retinoic acid on a DR4-containing promoter. In CHO cells that do not express endogenous RXR at as high a level as EC cells, cotransfection of RXR together with OR-1 is necessary to obtain induction by 9-*cis* retinoic acid. Thus acting as a helper of RXR, OR-1 appears to confer 9-*cis* retinoic acid signalling on DR4-containing promoters.

CLAIMS

1. An isolated nuclear receptor, having the amino acid sequence of Fig. 1 or substantially the same amino acid sequence as the amino acid sequence shown in Fig. 1 or an amino acid sequence functionally similar to that sequence.

2. A receptor according to claim 1 which is derived from rat.

3. A receptor according to claim 1 or 2 which binds to a DNA sequence comprising at least one repeat of the DNA sequence -AGGTCA-

4. A receptor according to claim 3 in which the DNA sequence comprises AGTCAGGTCACTCGAGGTCAGTCA.

5. A receptor according to any one of claims 1 to 4 which heterodimerizes with RXR to form a complex.

6. A complex comprising a receptor according to any preceding claim and RXR.

7. An amino acid sequence encoding a receptor according to claim 1 which is at least 90% identical with the amino acid sequence of Fig. 1.

8. An amino acid sequence according to claim 7 which is at least 95% identical

with the amino acid sequence of Fig. 1.

9. A DNA sequence encoding a nuclear receptor according to any one of claims 1 to 5 or a functionally similar nuclear receptor.

10. A DNA sequence according to claim 9 in which the DNA sequence is that given in Fig.2 or a DNA sequence substantially similar to that sequence.

11. The use of a nuclear receptor according to any one of claims 1 to 5 or a complex according to claim 6 or a ligand therefor in medicine.

12. The use of a nuclear receptor according to any one of claims 1 to 5 to modulate retinoic acid signalling *in vivo* or *in vitro*.

FIG. 1

```
   1  MSSPTSSLDT PLPGNGSPQP STSSTSPTIK EEGQETDPPP GSEGSSSAYI
  51  VVILEPEDEP ERKRKKGPAP KMLGHELCRV CGDKASGFHY NVLSCEGCKG
 101  FFRRSVVHGG AGRYACRGSG TCQMDAFMRR KCQLCRLRKC KEAGMREQCV
 151  LSEEQIRKKK IQKQQQQQPP PPTEPASGSS ARPAASPGTS EASSQGSGEG
 201  EGIQLTAAQE LMIQQLVAAQ LQCNKRSFSD QPKVTPWPLG ADPQSRDARQ
 251  QRFAHFTELA IISVQEIVDF AKQVPGFLQL GREDQIALLK ASTIEIMLLE
 301  TARRYNHETE CITFLKDFTY SKDDFHRAGL QVEFINPIFE FSRAMRRLGL
 351  DDAEYALLIA INIFSADRPN VQEPSRVEAL QQPYVEALLS YTRIKRPQDQ
 401  LRFPRMLMKL VSLRTLSSVH SEQVFALRLQ DKKLPPLLSE IWDVHE*
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FIG.2

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   1  CAAGTGCTGT GGAGGAGCAA TCACCGGTGC GGACACAGAG CTCCCGCCTC
  51  CCACAGCCAT TTCCAGGGTA ACGAAGTAGG AGACCCCCTC CTGCGACCCC
 101  CTCACGATCG CCGGTGCAGT CATGAGCCCC GCCTCCCCCT GGTGCACGGA
 151  GAGGGGCGGG GCCTGGAACG AGGCTGCTTC GTGACCCACT ATGTCTTCCC
 201  CCACAAGTTC TCTGGACACT CCCTTGCCTG GGAATGGTTC TCCCCAGCCC
 251  AGTACCTCCT CCACTTCACC CACTATTAAG GAGGAGGGAC AGGAGACTGA
 301  TCCACCTCCA GGCTCTGAAG GGTCCAGCTC TGCCTACATC GTGGTCATCT
 351  TAGAGCCAGA GGATGAACCT GAGCGCAAGC GGAAGAAGGG TCCGGCCCCG
 401  AAGATGCTGG GCCATGAGCT GTGCCGCGTG TGCGGGGACA AGGCCTCGGG
 451  CTTCCACTAC AATGTGCTCA GTTGTGAAGG CTGCAAAGGC TTCTTCCGGC
 501  GTAGCGTGGT CCATGGTGGG GCCGGGCGCT ATGCCTGTCG GGGCAGCGGA
 551  ACCTGCCAGA TGGATGCCTT CATGCGGCGC AAGTGCCAGC TCTGCAGACT
 601  GCGCAAGTGC AAGGAGGCTG GCATGCGGGA GCAGTGCGTG CTTTCTGAGG
 651  AGCAGATTCG GAAGAAAAAG ATTCAGAAGC AGCAACAGCA GCAGCCACCG
 701  CCCCCGACTG AGCCAGCATC CGGTAGCTCA GCCCGGCCTG CAGCCTCCCC
 751  TGGCACTTCG GAAGCAAGTA GCCAGGGCTC CGGGGAAGGA GAGGGCATCC
 801  AGCTGACAGC GGCTCAGGAG CTGATGATCC AACAGTTAGT TGCCGCGCAG
 851  CTGCAGTGCA ACAAGCGATC TTTCTCCGAC CAGCCTAAAG TCACGCCCTG
 901  GCCCTTGGGT GCAGACCCTC AGTCCCGAGA CGCTCGTCAG CAACGCTTTG
 951  CCCACTTCAC TGAGCTAGCC ATCATCTCAG TCCAGGAGAT CGTGGACTTC
1001  GCCAAGCAGG TGCCAGGGTT CCTGCAGCTG GGCCGGGAGG ACCAGATCGC
1051  CCTCCTGAAG GCATCCACCA TCGAGATCAT GTTGCTAGAG ACAGCCAGAC
1101  GCTACAACCA CGAGACAGAG TGCATCACGT TCCTGAAGGA CTTCACCTAC
```

FIG. 2 cont.

1151 AGCAAGGACG ACTTCCACCG TGCAGGCTTG CAGGTGGAGT TCATCAATCC

1201 CATCTTTGAG TTCTCTCGGG CTATGCGTCG GCTGGGCCTA GACGATGCAG

1251 AGTATGCCTT GCTCATTGCC ATCAACATCT TCTCAGCGGA CCGGCCTAAT

1301 GTGCAGGAGC CCAGCCGTGT GGAGGCTCTG CAGCAGCCCT ATGTGGAGGC

1351 CCTCCTCTCC TACACGAGGA TCAAGCGGCC GCAGGACCAG CTGCGCTTCC

1401 CACGAATGCT CATGAAGCTG GTGAGCCTGC GCACCCTCAG CTCCGTGCAC

1451 TCGGAGCAGG TTTTCGCATT GCGTCTCCAG GACAAGAAGC TGCCGCCTTT

1501 GCTGTCCGAG ATCTGGGATG TGCATGAGTA GGGGCCGCCA CAAGTGCCCC

1551 AGCCTTGGTG GTGTCTACTT GCAGATGGAC GCTTCCTTTG CCTTTCCTGG

1601 GGTGGGAGGA CACTGTCACA GCCCAGTCCC CTGGGCTCGG GCTGAGCGAG

1651 TGGCAGTTGG CACTAGAAGG TCCCACCCCA CCCGCTGAGT CTTCCAGGAG

1701 TGGTGAGGGT CACAGGCCCT AGCCTCTGAT CTTTACCAGC TGCCCTTCCT

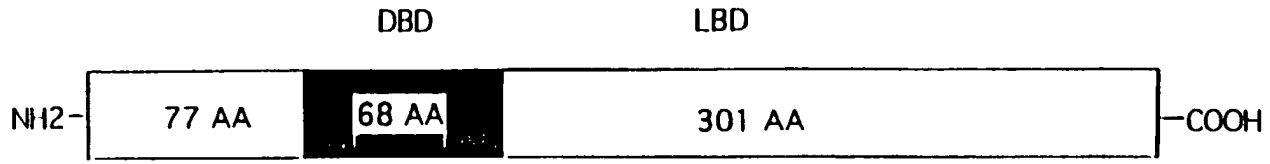
1751 CCCGAGCTTA CACCTCAGCC TACCACACCA TGCACCTTGA GTGGAGAGAG

1801 GTTAGGGCAG GTGGCTCCCC ACAGTTGGGA GACCACAGGC CCCCTCTTCT

1851 GCCCCTTTTA TTTAATAAAA AAATAAAATA AAAAAAAAAA AAAAAAAAAA

1901 AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAA

Figure 3



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h ARP-1	< 10 %	51.1 %	30 %
h COUP-TF	< 10 %	51.1 %	29 %
r VDR	< 10 %	50 %	34.7 %
h RXR α	< 10 %	50 %	23.6 %
r TR β	< 10 %	50 %	29.7 %
r PPAR	< 10 %	50 %	28.5 %
r MR	< 10 %	50 %	18.3 %
r NGF-1	< 10 %	48.5 %	30.3 %

FIG. 4

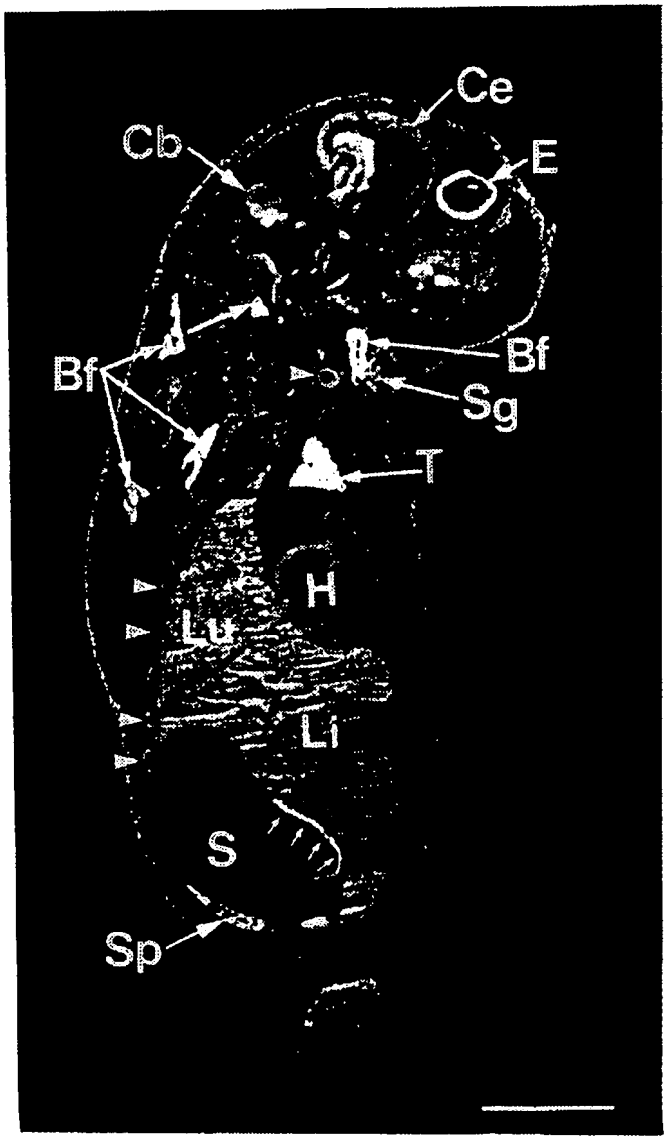


FIGURE 5A

DR-0 AGGTCA AGGTCA

DR-1 AGGTCA c AGGTCA

DR-2 AGGTCA cc AGGTCA

DR-3 AGGTCA ctc AGGTCA

DR-4 AGGTCA ctcg AGGTCA

DR-5 AGGTCA ctcgt AGGTCA

DR-6 AGGTCA ctcgtt AGGTCA

Figure 5 B

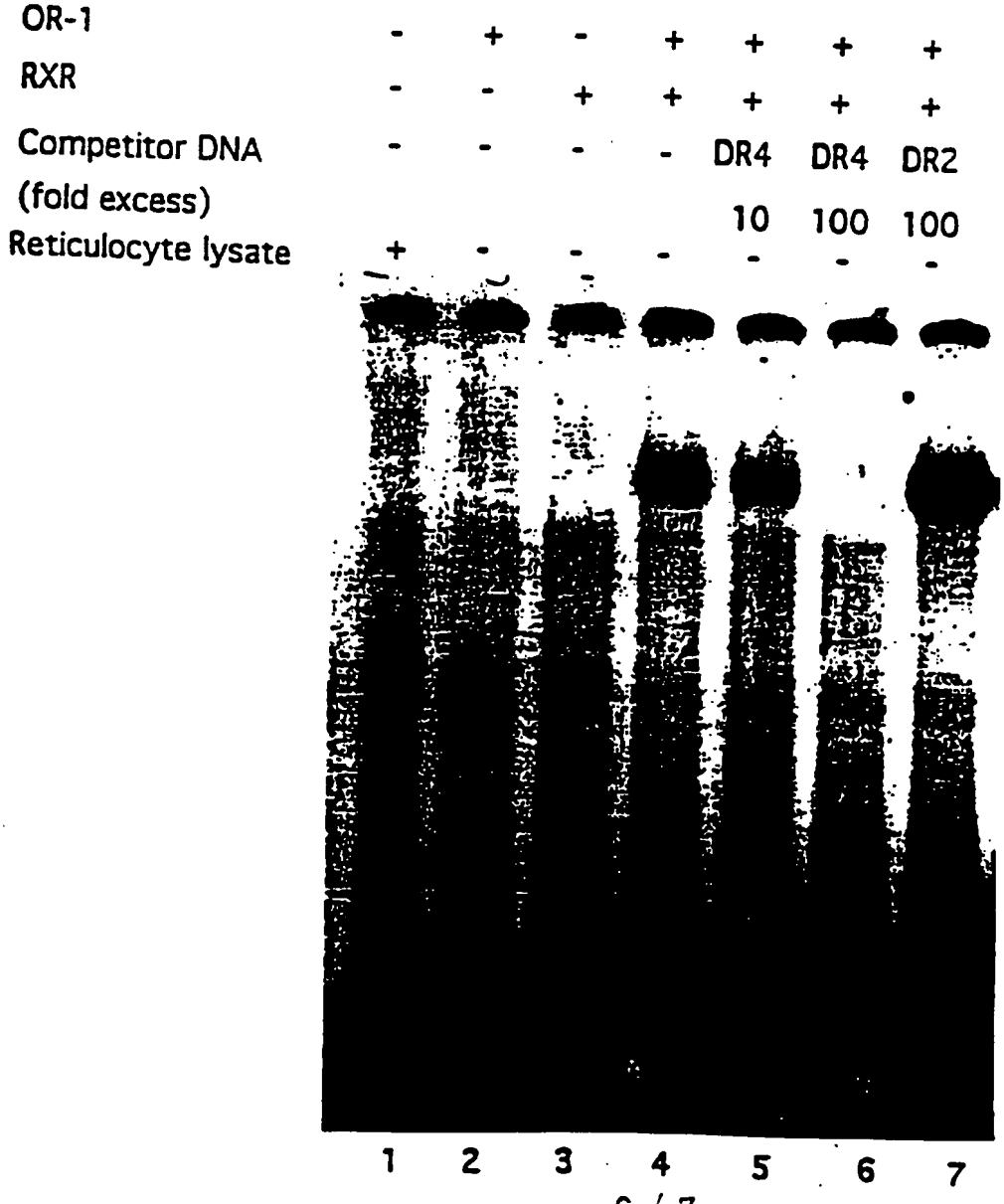
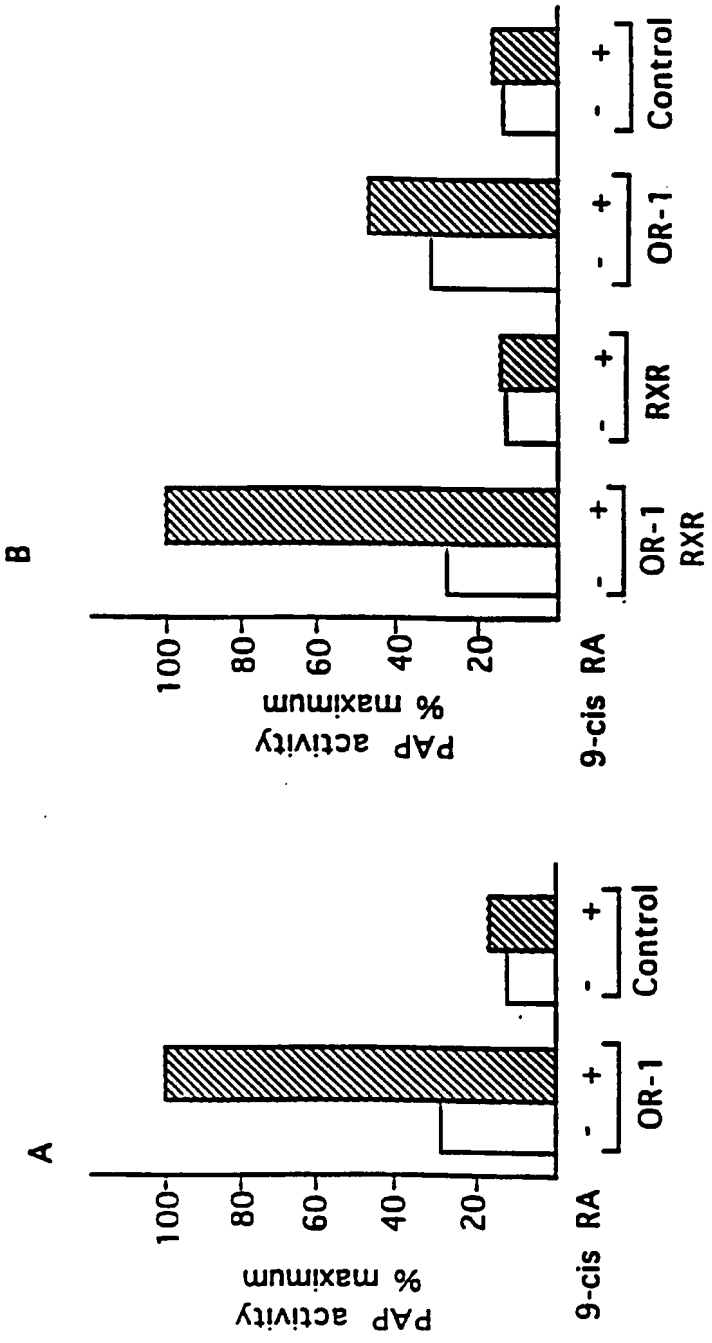


Figure 6


A
B
PAP activity
% maximum
100
80
60
40
20
9-cis RA
-
+
OR-1
RXR
Control